PRODUCT INFORMATION & ELISA MANUAL

Influenza A H3N2 Hemagglutinin Antibody Pair [HRP]

NBP2-79338

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked Immunosorbent Assay for quantitative detection of Influenza A Virus H3N2 Hemagglutinin.

For research use only.

Not for diagnostic or therapeutic procedures.

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BACKGROUND

Influenza (flu) is a respiratory infection in mammals and birds. This virus is divided into three main types (A, B and C). Influenza A is found in a wide variety of bird and mammal species and is further divided into subtypes based on differences in the membrane proteins hemagglutinin (HA) and neuraminidase (NA). H3N2 is a subtype of Influenza A. Hemagglutinin (HA) is a single-pass type I? integral membrane glycoprotein from the influenza virus, and comprises over 80% of the envelope proteins present in the virus particle. The HA is a trimer with a receptor binding pocket on the globular head of each monomer. In natural infection, inactive HA is matured into HA1 and HA2 outside the cell by one or more trypsin-like, arginine-specific endoprotease secreted by the bronchial epithelial cells. Binding of HA to sialic acid-containing receptors on the surface of its target cell brings about the attachment of the virus particle to the cell and forms a endosome. Low pH in endosomes induce an irreversible conformational change in HA2, releasing the hydrophobic portion “fusion peptide”. After which, virus penetrates the cell and pours its contents including the RNA genome into the cytoplasm mediated by fusion of the endocytosed virus particle’s own membrane and the endosomal membrane. Hemagglutinin plays a major role in the determination of host range restriction and virulence.

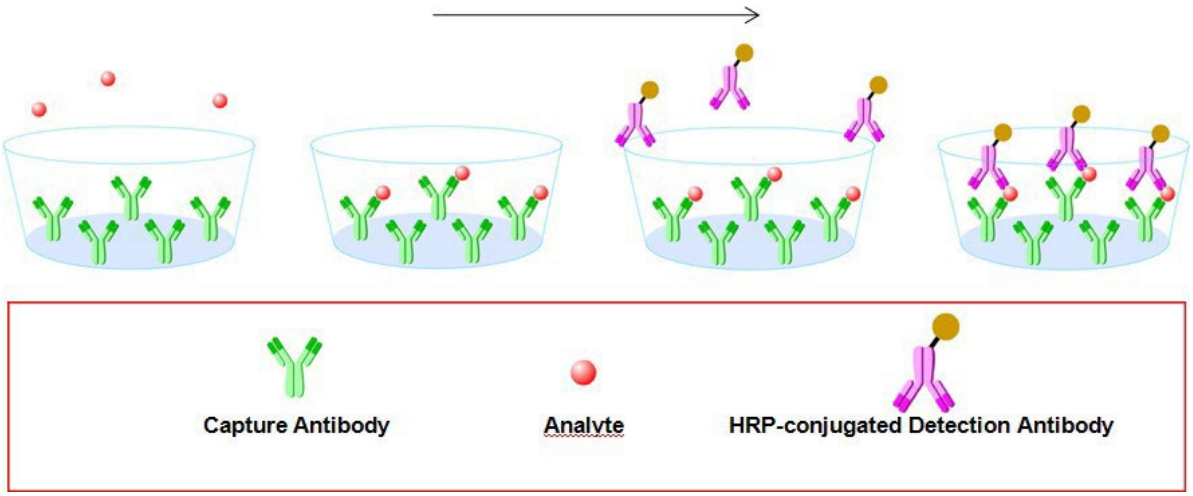
PRINCIPLE OF THE TEST

The Novus Biologicals Influenza A H3N2 Hemagglutinin Antibody Pair [HRP] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007) coated on a 96-well plate. Standards and samples are added to the wells, and any Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007) present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated mouse anti-Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007) monoclonal antibody is then added, producing an antibody- antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007) present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

INTENDED USE

- The Influenza A H3N2 Hemagglutinin Antibody Pair [HRP] is for the quantitative determination of Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007). It contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody – 1 mg/mL of rabbit Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007) HA monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 2 µg/mL in PBS before coating.

Detection Antibody – 0.2 mg/mL of mouse anti- Influenza A H3N2Hemagglutinin (A/Brisbane/10/2007) monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4℃). Dilute to working concentration of 0.8 µg/mL in detection antibody dilution buffer before use.

Standard – Each vial contains 1200 ng of recombinant Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007). Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 25000 pg/mL is recommended.

SOLUTIONS REQUIRED

PBS - 136.9 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, pH 7.4, 0.2 µm filtered

TBS - 20 mM Tris, 150 mM NaCl, pH 7.4

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na_2HPO_4 and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Store at 4℃ and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

Standard: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100μL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300μl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

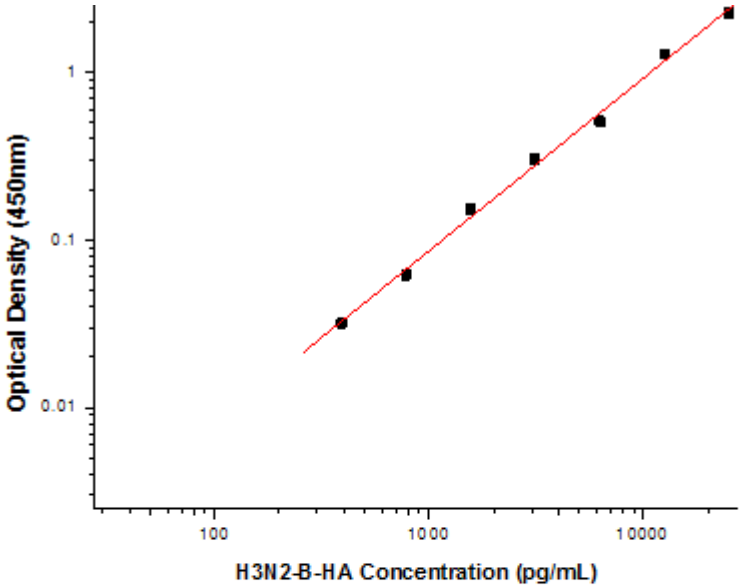
1. Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 μL of substrate solution to each well. Incubate for 20 minutes at room temperature (**if substrate solution is not as requested, the incubation time should be optimized**). Avoid placing the plate in direct light.
6. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/mL)	Zero standard subtracted OD
0	0
390. 63	0. 032
781. 25	0. 062
1562. 5	0.152
3125	0.304
6250	0.510
12500	1.274
25000	2.243

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of Influenza A H3N2 Hemagglutinin (A/Brisbane/10/2007) was determined to be approximately **390.63 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

SPECIFICITY

The following factors were assayed and exhibited **cross-reactivity** with

H3N2 (A/Wisconsin/67/X-161/2005) HA

H3N2 (A/Wyoming /3/2003) HA

H3N2 (A/Aichi/2/68) HA

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
		Try higher dilution rate of samples

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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Notes